# Negative-pressure wound therapy: A hemostatic adjunct for control of coagulopathic hemorrhage in large soft tissue wounds

**Bijan S. Kheirabadi, PhD, Irasema B. Terrazas, MS, James F. Williams, MPAS, Margaret A. Hanson, DVM, Michael A. Dubick, PhD,** *and* **Lorne H. Blackbourne, MD,** *Fort Sam Houston, Texas*

**BACKGROUND:** Negative-pressure wound therapy has been commonly used for treating chronic wounds and recently applied for treatment of traumatic wounds. We investigated the potential hemostatic benefit of negative-pressure wound therapy for control of refractory hemorrhage in a soft tissue wound model in swine.

**METHODS:** Coagulopathy was induced in pigs (n 38, 36 kg) by hemodilution and hypothermia. Next, a large soft tissue wound (diameter, approximately 20 cm) was created by slicing the gluteus maximus muscle. Free bleeding was allowed for 1 minute, and wounds were then randomly dressed with either laparotomy gauze (G) alone or TraumaPad (TP, a kaolin-coated dressing) alone or in combination with negative pressure (NP, approximately 500 mm Hg). All wounds were sealed with adhesive drapes. Fluid resuscitation was administered and targeted to mean arterial pressure of 60 mm Hg. Pigs were observed for 150 minutes or until death after which tissues were sampled for histologic examination.

**RESULTS:** Induced coagulopathy as measured by increases in prothrombin time (12%) and activated partial thromboplastin time (22%) and decreases in fibrinogen (48%) were similar in all groups. There were no differences in initial bleeding rates (4.5 mL/kg/min). Dressing the wounds with G or TP produced hemostasis only in one pig (1 of 18 pigs). Addition of NP to these dressings secured hemostasis in 70% (G) and 90% (TP) of animals with average hemostasis time of 34 minutes and 25 minutes, respectively. Blood losses and fluid resuscitation requirements were significantly less, and survival times were significantly longer in NP adjunct groups than in the other groups. Survival rates were 80% (G+NP) and 90% (TP+NP) versus 0% (G) and 10% (TP) in the respective groups. Histologic examination showed similar superficial myofibril damages in all groups.

**CONCLUSION:** To our knowledge, the present data provide the first evidence that NP serves as an effective hemostatic adjunct and when combined with standard hemostatic dressing it is able to stop lethal coagulopathic bleeding in large soft tissue wounds. (*J Trauma Acute Care Surg*. 2012;73: 1188 1194. Copyright © 2012 by Lippincott Williams & Wilkins)

**KEY WORDS:** Negative-pressure wound therapy; hemostatic adjunct; coagulopathy; swine.

Negative-pressure wound therapy (NPWT) is a treatment modality that has become widely adapted for a broad range of wound indications. It was originally developed in the 1990s for the management of chronically infected wounds and has more recently been used for the treatment of traumatic wounds.[1,2] NPWT is a generic technology, which can be applied to a wound using a range of variables including source and level of negative pressure, wound filter, and wound contact layer. This modality creates a wound environment of subatmospheric pressure (−50 to −200 mm Hg) that promotes healing of acute or chronic wounds, reduces infection, and enhances healing of burn injuries. While the exact mechanism of action is not yet fully understood, NPWT is thought to benefit wound healing by (1) removing interstitial fluid that contains desiccated tissue and inhibitory factors such as collagenases and inflammatory cytokines, (2) decreasing the level of bacteria, (3) improving blood flow in the wound bed and surrounding tissue, (4) enhancing angiogenesis, (5) promoting granulation tissue, and (6) pulling the wound edges together and stimulating faster cell growth. The dynamic interplay of these actions is thought to improve wound healing.[3–5]

The potential benefits of NPWT for treating traumatic wounds such as large soft tissue injuries, high-energy penetrating trauma and open fractures have also been reported.[6–10] NPWT has been used for evacuating serous drainage or hematoma/blood from acute wounds, thereby reducing the risk of postoperative infections.[7,11–13]

We investigated the potential hemostatic benefits of NPWT using strong negative pressure (NP) in a large soft tissue wound model in pigs that mimicked explosion injures. The hemorrhage from such wound model was easily controlled when the wounds were dressed with regular gauze and sealed with adhesive drapes (requiring no other adjunct). Hence, a preexisting coagulopathic condition was produced in pigs before the injury to create refractory bleeding to examine potential hemostatic effect of NPWT.

## MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All

Submitted: May 3, 2012, Revised: July 17, 2012, Accepted: July 17, 2012.
From the US Army Institute of Surgical Research, Fort Sam Houston, Texas.
This study was presented as a poster at 25th annual meeting of Eastern Association for the Surgery of Trauma , January 10-14, 2012, in Lake Buena Vista, Florida.
The opinions or assertions expressed herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of Defense.
Address for reprints: Bijan S. Kheirabadi, PhD, 3650 Chambers Pass, BHT2, Bldg 3610, Fort Sam Houston, TX 78234; email: BIJAN.KHEIRABADI@US.ARMY.MIL.

DOI: 10.1097/TA.0b013e31826f98ea

# Report Documentation Page

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| **01 NOV 2012** | **N/A** | **-** |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| **Negative-pressure wound therapy: A hemostatic adjunct for control of coagulopathic hemorrhage in large soft tissue wounds** | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
| **Kheirabadi B. S., Terrazas I. B., Williams J. F., Hanson M. A., Dubick M. A., Blackbourne L. H.,** | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| **United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX** | |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

12. DISTRIBUTION/AVAILABILITY STATEMENT
**Approved for public release, distribution unlimited**

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a REPORT | b ABSTRACT | c THIS PAGE | | | |
| **unclassified** | **unclassified** | **unclassified** | **UU** | **7** | |

animals received care and were used in strict compliance with *The Guide for the Care and Use of Laboratory Animals*.[14]

Two laparotomy sponges (12 × 12 inches) were used as controls for packing the wound. These were covered with a large sterile adhesive drape (3M Ioban, St. Paul, MN) that was tightly wrapped around the leg. For hemostatic treatment, a hemostatic dressing called TraumaPad (Z-Medica Corp., Wallingford, CT) was used that was generously donated by Combat Medical Systems (Fayetteville, NC). This dressing has a composition similar to Combat Gauze (surgical gauze coated with kaolin minerals) but is customized for treating large wounds. The size and shape of TraumaPad was similar to that of the lap sponge. For treating wounds, one intact TraumaPad was placed on the wound and covered with a lap sponge. These dressings were held in place by wrapping them with adhesive drape that sealed the wound. This was performed to replicate conditions that were required for applying NP. For NP treatment, necessary supplies such as wound filter foam (GranuFoam) and pressure assembly line (Sensa T.R.A.C. Pad) were purchased from KCI in San Antonio, Texas. To apply NP, a precut foam fitted to the wound size was placed either directly on injured tissues (preliminary tests) or on the lap sponges with or without TraumaPad and then sealed in place by wrapping them with the adhesive drape. The NP assembly line, which was connected to operating room suction line, was then attached to the foam through a hole on the drape.

## Animal Preparation and Surgical Procedures

Yorkshire castrated male pigs (34–40 kg) were purchased from Midwest Research Swine (Gibbon, MN). Upon arrival, the animals were housed and observed for 5 days to exclude the possibility of preexisting disease by blood testing and allow acclimation. Pigs were fasted for 12 hours to 18 hours before surgery with free access to water. On the day of surgery, pigs were induced, intubated, and anesthetized by inhalation anesthesia (1–2% isoflurane) and mechanically ventilated as described previously.[15] Maintenance fluid (Lactated Ringer's solution) was administered at 5 mL/kg per hour intravenously.

Common carotid artery, external jugular vein, and femoral artery were cannulated via cutdowns, and baseline blood samples were collected from the arterial line for complete blood count, coagulation (prothrombin time [PT], activated partial thromboplastin time [aPTT], and fibrinogen), and arterial blood gas analysis. Following a midline laparotomy, infrarenal aorta was exposed, and a plastic loop was loosely placed around the aorta. The loop ends were externalized and abdomen was closed.

Next, coagulopathy was produced in pigs by 50% hemodilution and mild hypothermia (34.5°C body temperature), as previously described.[16] Following these procedures, additional blood samples (before injury) were collected for complete blood count, arterial blood gas, and coagulation measurements. A stable mean arterial pressure (MAP) of 60 mm Hg or higher was required to proceed.

To produce the injury, the perimeter of a 16-cm diameter round wound was marked on the pig's buttock muscle. Next, the abdominal aorta was occluded temporarily by tightening the vascular loop to prevent bleeding during laceration. The skin was then cut and removed from the marked area exposing the gluteus maximus muscle. Using a special blade (Sakura Finetek USA, Torrance, CA), the muscle was sliced along the edge of the wound (Fig. 1*A*), and the layers were removed to approximate 1% of pig's body weight. The aortic loop was then released, and 1 minute of unrestricted bleeding was allowed

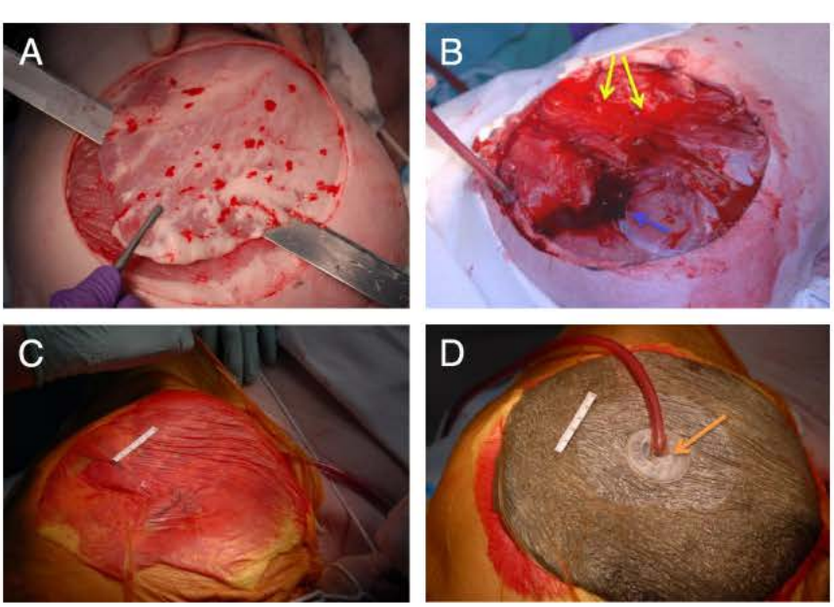


**Figure 1.** Swine large soft tissue model. The wound was created by slicing approximately 400-g tissue from gluteus maximus muscle (*A*) resulting in refractory hemorrhage from multiple small vessels injuries (*arrows*, *B*). Wounds were subsequently dressed with TraumaPad/gauze alone (*C*) or TraumaPad/gauze + NP application (*D*); the *arrow* refers to pressure assembly line.

(Fig. 1*B*). Shed blood during this period was collected to measure pretreatment blood loss, indicator of injury severity and reproducibility of hemorrhage.

Next, wounds were rapidly dressed and treated in a randomized fashion with gauze alone or TraumaPad alone (Fig. 1*C*) or with these dressings plus NP that was applied continuously (Fig. 1*D*). The NP source was the operating room suction line (approximately −500 mm Hg) that was used after more standard levels of NP for wound healing were found ineffective. The foam was not used in the standard treatments (gauze alone or TraumaPad alone) because it would decrease tamponade effect and likely increase the blood loss. To meet compression requirements, the gauze and TraumaPad dressings were pressed against the wounds by placing a 10-lb weight plate on them for 3 minutes. After compression of control dressings and applying NP to treatment wounds, fluid resuscitation was administered (Hextend, up to 3 L at 50 mL/min) intravenously to raise and maintain MAP of 60 mm Hg or greater during experiment. Any shed blood during this period was collected and measured as posttreatment blood loss. Animals were monitored for 2.5 hours or until death as determined previously.[16] Final blood samples were collected, and animals were euthanized. Tissue samples were collected from the center and the edge of the wounds for histologic analysis. The hematoxylin-eosin–stained histologic slides were coded and then examined by our veterinary pathologist (M.A.H.) who was blinded to the treatment group. Once the subjective analysis was complete, samples were identified; the results were categorized and compared among treatment groups.

In experiments with NP, hemostasis was checked at the conclusion of experiments before the animals were euthanized. In these instances, the suction line was disconnected, the adhesive drape was cut off, and foam and dressings were carefully (layer by layer) removed from the wound. The status of bleeding/hemostasis was observed for a few minutes.

## Data Analysis

Data were analyzed by one-way analysis of variance (ANOVA, parametric data), Kruskal-Wallis test (nonparametric), $\chi^2$, and the log-rank for statistical comparisons. Bigroup comparisons were performed by using the Tukey's and Dunnett's tests. A $p < 0.05$ was considered statistically significant.

# RESULTS

A few pilot experiments (n = 2) were initially performed in which the NP was applied to the bleeding wound in a similar manner as practiced for treating chronic wounds (low NP [−125 mm Hg] and no dressing). The results clearly showed that such an application method has no hemostatic effect and might even increase the hemorrhage. In subsequent pilot experiments (n = 3), a stronger NP was applied (−480 mm Hg to −520 mm Hg) continuously for the duration of each experiment and added to standard hemostatic treatment as an adjunct. Applying high suction pressure immediately flattened and compressed the foam and dressings against damaged tissues. Any accumulated blood was rapidly evacuated, and additional blood losses were continuously removed until hemostasis was achieved. These findings were encouraging and justified design of the main study as described later.

Thirty eight pigs, divided randomly into four groups (n = 8 or 10) were used in the main study. Baseline values for hemodynamic, temperature, and hematologic measures were within reference ranges with no significant differences among groups (Table 1). As expected, isovolemic hemodilution (approximately 50%) with Hextend reduced hemoglobin concentration and platelet counts by approximately 60% and fibrinogen concentration by 48% in all animals. The PT and aPTT, however, were prolonged only by 12% and 22%, respectively. A small decreased in MAP (approximately 6.5 mm Hg) was measured after hemodilution and hypothermia in some animals, but

**TABLE 1.** Physiologic and Hematologic Measures of Pigs at Baseline and at Preinjury (After Hemodilution and Hypothermia) Time Points

| | Combined Groups (n = 38) | Gauze (n = 8) | TraumaPad (n = 10) | Gauze + NP (n = 10) | TraumaPad + NP (n = 10) | Overall |
|---|---|---|---|---|---|---|
| **Values, Mean (SEM)** | **Baseline** | **Preinjury** | **Preinjury** | **Preinjury** | **Preinjury** | ***p*** |
| Temperature, °C | 37.5 (0.23) | 34.6 (0.12) | 34.7 (0.08) | 34.6 (0.07) | 34.7 (0.08) | 0.78 |
| MAP, mm Hg | 72.6 (2.0) | 65.6 (2.05) | 64.6 (1.7) | 69.4 (2.6) | 63.8 (0.8) | 0.20 |
| Hemoglobin level, g/dL | 10.4 (0.12) | 4.6 (0.23) | 4.3 (0.18) | 4.5 (0.17) | 4.4 (0.13) | 0.50 |
| Hematocrit, % | 30.7 (0.34) | 13.8 (0.67) | 13.0 (0.47) | 13.7 (0.49) | 13.2 (0.38) | 0.48 |
| White blood cell, $\times 10^9$/L | 17.9 (0.56) | 6.42 (0.53) | 7.04 (0.72) | 7.13 (0.59) | 6.9 (0.38) | 0.82 |
| Platelet, $\times 10^9$/L | 411.4 (17.1) | 135.5 (10.6) | 163.9 (13.7) | 162.5 (16.9) | 152.6 (12.9) | 0.64 |
| PT, s | 11.8 (0.25) | 13.5 (0.42) | 13 (0.33) | 13.4 (0.58) | 13.2 (0.27) | 0.73 |
| aPTT, s | 17.0 (0.36) | 22 (0.99) | 19.5 (0.7)5 | 21.2 (1.13) | 20.4 (1.19) | 0.45 |
| Fibrinogen, mg/dL | 254.2 (6.8) | 113.9 (13.0) | 138.7 (17.8) | 123.8 (11.4) | 149.4 (14.6) | 0.23 |
| pH | 7.4 (0.01) | 7.44 (0.01) | 7.4 (0.01) | 7.44 (0.01) | 7.4 (0.01) | 0.35 |
| Lactate, mM | 1.3 (0.08) | 2.15 (0.15) | 1.8 (0.12) | 1.9 (0.12) | 1.8 (0.1) | 0.28 |
| Base excess, mM | 4.7 (0.23) | 5.2 (0.49) | 4.6 (0.51) | 5.6 (0.44) | 5.2 (0.38) | 0.11 |
| iCa, mM | 1.4 (0.01) | 1.34 (0.01) | 1.3 (0.02) | 1.36 (0.02) | 1.3 (0.02) | 0.90 |

iCa, ionized calcium.

Data are expressed as mean (SEM) and analyzed by one-way ANOVA test. No significant difference was found in the baseline measurements among groups; therefore, the averages for all animals are listed in the table. The *p* values in the table represent comparisons of measurements after hemodilution and hypothermia (preinjury) among groups.

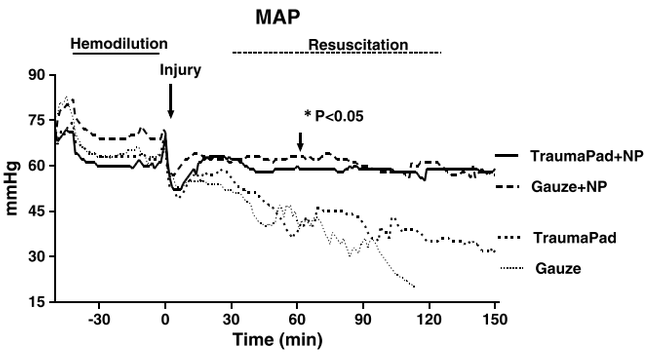


**Figure 2.** The average MAP of treated pigs during the experiments. Note the pressure drop after muscular injury/hemorrhage and return to baseline in NP-treated animals. The number of animals contributing to each average MAP decreases with time as pigs died at different time points.

this change was not statistically significant. There were no differences in MAP, hypothermia, and laboratory results after coagulopathy induction among groups (Table 1, preinjury values).

Muscle tissues (332–424 g) were excised from all pigs with no significant difference among groups. This amount of tissue constituted approximately 1% (0.99 [0.08]) of their body weights. Following injury and start of hemorrhage, MAP dropped approximately 10 mm Hg for the first 2 minutes to 3 minutes until wounds were dressed and fluid resuscitation started (Fig. 2). The injury and hemorrhage were reproducible and resulted in an average of 4.5-mL/kg per minute blood loss during the initial 1-minute free bleeding period. Dressing the wounds with gauze or TraumaPad took approximately 2 minutes but produced hemostasis only in one experiment (TraumaPad treated) after 95 minutes of slow bleeding. Addition of NP to these dressings, however, stopped hemorrhage in 80% (16 of 20) of pigs with an average hemostasis time of 34 minutes and 25 minutes for gauze- and TraumaPad-dressed groups, respectively (Table 2).

Infusion of Hextend restored and stabilized the MAP at target level in dressing + NP groups but was not effective in dressing-alone groups because of continued hemorrhage (Fig. 2). At 60 minutes after injury, MAP of NP-treated animals was significantly higher than that of the dressing-alone groups ($p < 0.05$). The total volume of fluid administered to achieve target pressures were significantly less ($p < 0.05$) in dressing + NP-treated groups than in groups treated with dressing alone (Table 2). Concurrently, posttreatment blood loss was significantly less in dressing + NP-treated groups than in the dressing-alone group (Table 2). In addition, the final values for hemoglobin, fibrinogen, and white blood cell and platelet counts were higher in NP groups than in the dressing-alone groups (Table 3). The clotting times were also shorter, and shock indices (lactate and base excess) were closer to the baseline (preinjury) levels in NP groups than in the other animals (Table 3). No significant difference was found between dressing the wound with regular gauze or TraumaPad with or without NP addition; however, a trend in favor of TraumaPad was apparent.

The pigs' survival rates coincided with hemostasis achievement except in one case (Gauze + NP group) where bleeding continued at a slow rate but the animal survived the duration of the experiment (2.5 hours). The survival time analysis and the advantage of application of NP are shown in Figure 3. Inspection of the wounds after successful treatment with NP showed significant decrease in bleeding (slow oozing) of the injured tissues after removal of NP and dressings.

Histologically, all samples exhibited variable amounts of acute degeneration and inflammation. The changes included cellular swelling, inflammation, splitting of muscle fibers, vacuolization of sarcoplasm, fiber fragmentation, and edema. The depth of histologic evident damages did not exceed 1.5 mm in all groups, and based on a subjective evaluation, no significant treatment effects were apparent. Examination of slides under polarized light detected kaolin residues in two specimens, which were seen only on the surface of the wound.

## DISCUSSION

The potential for hemostatic effects of NPWT was initially suspected by our surgeon colleagues who were operating on burn injuries with bleeding and used NPWT to promote wound healing. This study was therefore designed to investigate possible hemostatic benefit of NPWT for treating coagulopathic hemorrhage in large soft tissue wounds that often cannot be managed efficiently with standard dressing or surgical intervention. For this purpose,

**TABLE 2.** Outcomes of Treating Hemorrhage in Large Soft Tissue Wounds in Coagulopathic Swine

| Values, Mean (SEM) | Gauze (n = 8) | TraumaPad (n = 10) | Gauze + NP (n = 10) | TraumaPad + NP (n = 10) | Overall *p* |
|---|---|---|---|---|---|
| Hemostasis achieved (final) | 0/8 | 1/10 | 7/10 | 9/10 | <0.0001 |
| Time to Hemostasis, min | N/A | 95* | 34.4 (8.03)* | 25.1 (9.19)* | |
| Pretreatment blood loss, mL/kg | 4.1 (0.5) | 4.5 (0.4) | 4.1 (0.6) | 5.1 (0.4) | >0.1 |
| Posttreatment blood loss, mL/kg | 95.1 (11) | 97 (9.1) | 33 (13.3)† | 19 (8.4)† | <0.0001 |
| Resuscitation fluid, mL/kg | 72.6 (5.3) | 70.2 (6.3) | 25.3 (9.3)‡ | 17.9 (7.1)† | 0.0008 |
| Survival time, min | 70.7 (7.8) | 91 (12.95) | 145.3 (4.21)† | 146.3 (3.66)† | <0.0001 |
| Percent survival | 0 | 10 | 80† | 90† | <0.0001 |

*These data represent only the results of the successful (hemostasis achieved) experiments.
†$p < 0.05$ versus gauze or TraumaPad.
‡$p < 0.05$ versus gauze.
Data are expressed as mean (SEM) and analyzed by $\chi^2$, log-rank, and one-way ANOVA tests.
N/A, time to hemostasis could not be determined since no hemostasis was achieved in this group, and for that reason, statistical comparison among groups could not be performed.

**TABLE 3.** Final Hematologic Measurements of the Coagulopathic Pigs Following Hemostatic Treatment

| Values, Mean (SEM) | Gauze (n = 8) Final | TraumaPad (n = 10) Final | Gauze + NP (n = 10) Final | TraumaPad + NP (n = 10) Final | Overall *p* |
|---|---|---|---|---|---|
| Temperature, °C | 34.4 (0.3) | 34.4 (0.1) | 34.6 (0.2) | 34.7 (0.1) | 0.49 |
| MAP, mm Hg | 15.3 (1.3) | 19.6 (4.2) | 52.1 (6.8)* | 54.2 (4.5)* | 0.0004 |
| Hemoglobin level, g/dL | 1.2 (0.2) | 2.06 (0.4) | 4.7 (0.7)* | 4.6 (0.3)† | 0.001 |
| Hematocrit, % | 4.41 (0.7) | 6.83 (1.2) | 14.03 (2.0)† | 13.6 (0.8)† | 0.003 |
| White blood cell, $\times 10^9$/L | 2.4 (0.4) | 3.5 (1.2) | 9.7 (1.5)† | 11.3 (1.2)† | 0.004 |
| Platelet, $\times 10^9$/L | 47.5 (12.9) | 77.0 (18.6) | 179.6 (37.4)† | 197.6 (24.2)† | 0.002 |
| PT, s | 26.9 (2.6) | 27.3 (2.4) | 17.1 (2.2)† | 15.4 (1.4)† | 0.007 |
| aPTT, s | 41.7 (2.6) | 44.6 (4.0) | 30.6 (4.9) | 24.9 (2.2) | 0.03 |
| Fibrinogen, mg/dL | N/A | 92.9 | 112.7 (8.7) | 129.6 (15.8) | |
| pH | 7.53 (0.07) | 7.5 (0.03) | 7.48 (0.02) | 7.5 (0.01) | 0.7 |
| Lactate, mM | 6.61 (0.8) | 6.2 (0.9) | 2.6 (0.9)† | 1.1 (0.3)* | 0.002 |
| Base excess, mM | 1.5 (1.5) | 1.7 (1.2) | 5.8 (1.1)† | 6.8 (0.5)† | 0.002 |
| iCa, mM | 1.40 (0.02) | 1.38 (0.02) | 1.33 (0.01)† | 1.32 (0.01)† | 0.005 |

*$p < 0.05$ versus gauze or TraumaPad.
†$p < 0.05$ versus gauze.
Data are expressed as mean (SEM) and analyzed by one-way ANOVA test.
Final blood samples were collected from all the animals (survivors and nonsurvivors) before euthanasia.
N/A, fibrinogen could not be measured in the final blood samples of this group (nonsurvivors) because of excessive hemodilution and therefore could not be compared with other groups. iCa, ionized calcium.

we developed a combat-relevant large soft tissue wound model (as seen in improvised explosive device explosions) with multiple bleeding sites in coagulopathic pigs. The bleeding from such wounds could not be controlled with standard or an advanced hemostatic dressing with kaolin (TraumaPad). Applying routine NPWT (−100–150 mm Hg NP alone), as used for treating chronic or burn wounds, was also found to be ineffective to control hemorrhage in this model (pilot experiments). This result was not unexpected if one considers that applying negative pressure will draw/pool more blood to the injured surface tissues and likely will increase hemorrhage. Concurrently, a potential adverse effect of NPWT was reported to be increased risk of bleeding particularly for patients on anticoagulant therapy.[17]

In our subsequent experiments, the NPWT was examined as an adjunct to standard hemostatic treatment, and stronger NP was used. Furthermore, this strategy was compared with treating the wound with TraumaPad, a large version of Combat Gauze that is the current standard for treating external hemorrhage in the US Military. Combat Gauze dressing was found to be an effective hemostat against arterial bleeding in normal pigs[15] but had limited efficacy to control hemorrhage in coagulopathic animals.[16] The results from this study confirmed previous findings and showed poor efficacy of this dressing as well as regular gauze to control coagulopathic bleeding even in wounds with small vessels injuries. Application of strong NP to these wounds dressed with standard gauze or TraumaPad, however, significantly improved hemostasis and prevented exsanguination that occurred in most of the animals treated with dressings alone. The higher survival rates were caused by more effective hemorrhage control (less blood loss), lower fluid resuscitation, and better blood pressure maintenance and tissue perfusion (lower shock indices). Histologic assessment of treated muscle tissues showed no acute adverse effect of negative pressure application in this model. However, applying strong NP for longer periods or on more sensitive tissues such as blood vessels or nerves may have some adverse effects that should carefully be investigated. Histologic examination of the tissues was not performed in the earlier pilot tests when −125 mm Hg NP was tested since no hemostatic efficacy was observed in those experiments.

The exact mechanism by which NP promoted hemostasis in our experiments is unknown. However, it may be speculated based on our final observations. We noticed that in the successful experiments, hemorrhage had completely stopped, and no rebleeding occurred even after cessation of NP and restoration of atmospheric pressure on the wound. Subsequently, when dressings were removed from the wound, only minor rebleeding started although blood was more diluted at this time and blood pressure was near baseline. The rebleeding may have been caused

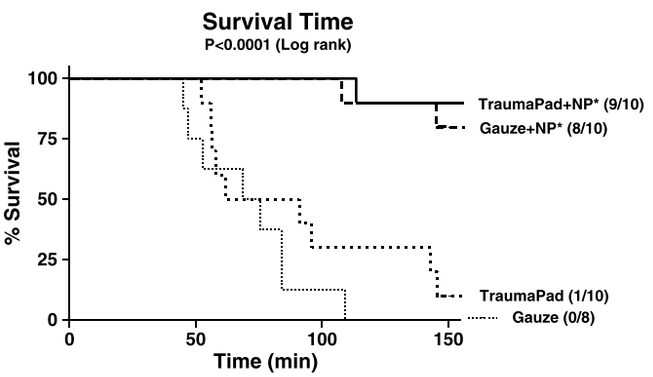


**Figure 3.** Kaplan-Meier analysis of survival time of pigs following injuries and treatments. Addition of NP to dressing treatment of the wounds significantly increased the survival time ($p < 0.05$).

by disruption of the blood clots formed in conjunction with dressings as the dressings were removed. However, since the rebleeding was insignificant, it is also implied that the strong NP may have constricted/occluded small blood vessels and thereby mechanically reduced the hemorrhage. Moreover, this constrictive effect apparently persisted even after NP was discontinued. The compression force of NP also strongly apposed dressings to wound surface favoring clot formation by the gauze. The two mechanisms together fully stopped hemorrhage while neither mechanism alone (NP force or gauze clotting ability) was able to stop the coagulopathic bleeding. In a few cases in which NP and dressings application did not secure hemostasis, the reason seemed to be the inability of blood/dressing to form clot and seal the injuries, rather than failure in applying and maintaining of NP on the wound.

Although NPWT was initially developed for treating chronic nonhealing wounds, greater experience in civilian and combat settings have extended indication of this technology for management of acute injuries. The conditions that seem to benefit from NPWT include control of infection and limb salvage,[7,18] skin graft success,[19,20] fasciotomy[21] and wound temporizing as a bridge to definitive treatment.[11,22,23] In the current military conflict, explosion force of improvised explosive devices has produced devastating combat wounds presenting new challenges to the military health care providers. An example is the large soft tissue wound, also known as the "shark bite," in the extremities and trunk, with massive destruction/loss of soft and hard tissues. Bleeding from injured large vessels associated with these wounds is controlled surgically, but the diffuse bleeding from multiple small vessel injuries caused by loss of skeletal muscle is much harder to manage with surgical interventions. The use of electrical cautery causes more tissue damage and delays healing, but covering the wounds with gauze or hemostatic dressing is mostly ineffective because of coagulopathy that often develops in these patients.[24] The result is persistent bleeding that requires frequent changes of dressings and transfusion of more blood and blood products. The use of NPWT is likely to benefit the management and treatment of these complex injuries and reduce blood loss, reduce transfusion requirement, and shorten hospital stays.

There are several limitations in this study that should be described. This study showed hemostatic potential of NPWT in a relatively simple superficial wound that was easily dressed and subjected to a uniformly distributed negative pressure. It remains to be seen how this technology may benefit control of hemorrhage in more complex traumatic wounds for patients. The tight sealing of the wound is also a critical step in achieving hemostatic advantage of NPWT. This requirement was met in this experimental study, but it might be harder to accomplish when a more complex wound is being treated in a clinical (less controlled) environment. Although the optimum negative-pressure force for hemostatic action of NPWT is unknown and it may vary depending on the size and complexity of the wounds, it was apparent from our experiments that strong negative pressure (500 mm Hg or more) was needed to obtain the full benefit of NPWT. This requirement may preclude the use of this technology in some circumstances. We did not determine the effectiveness of using lower NP (e.g., 300–400 mm Hg) for achieving hemostasis in this model and also did not determine the least effective pressure. This would have required additional work that was beyond the scope of the present study.

In conclusion, to our knowledge these data provide the first evidence that NP acts as an effective hemostatic adjunct and, when combined with standard hemostatic dressing, is able to stop lethal coagulopathic bleeding in large soft tissue wounds. This effect is likely caused by sustained compression of damaged tissues that constricts small blood vessels and promotes clot formation in conjunction with apposition of the dressings.

## AUTHORSHIP

All authors were involved in the design of the study, methodology development, and preparation and editing of the article. B.S.K. and I.B.T. performed the surgical procedures, treatments, data collection and analysis. J.F.W. provided the NPWT training. M.AH., our veterinarian pathologist, performed the histologic analysis.

## ACKNOWLEDGMENTS

We express our sincere gratitude to Dr. Harold Klemcke for his careful review and editorial assistance of the article. We also acknowledge our Veterinary Support Branch for their support and assistance in conducting these experiments. The excellent technical assistance by Dr. Adekoye Sanni (DVM), SPC Birk Greene, and SPC Alice Craig is greatly appreciated.

## DISCLOSURE

The funding for this work was provided solely by the US Army Medical Research and Materiel Command.

## REFERENCES

1. Argenta LC, Morykwas MJ. Vacuum-assisted closure: a new method for wound control and treatment. Clinical experience. *Ann Plast Surg*. 1997;38:563.
2. Xie X, McGregor M, Dendukuri N. The clinical effectiveness of negative pressure wound therapy: a systemic review. *J Wound Care*. 2010;19: 490 495.
3. Banwell PE, Musgrave M. Topical negative pressure therapy: mechanisms and indications. *Int Wound J*. 2004;1:95 106.
4. Morykwas MJ, Argenta LC, Shelton-Brown EI, et al. Vacuum-assisted closure: a new method for wound control and treatment. Animal studies and basic foundation. *Ann Plast Surg*. 1997;38:553 561.
5. Saxena VS, Hwang C, Huang SM, et al. Vacuum-assisted closure: microdeformations of wounds and cell proliferation. *Plast Reconstr Surg*. 2004;114:1086 1096.
6. Pirela-Cruz MA, Machen MS, Esquvel D. Management of soft-tissue wounds with negative pressure therapy lessons learned from the war zone. *J Hand Ther*. 2008;21:196 203.
7. Leininger BE, Rasmussen TE, Smith DL, et al. Experience with wound VAC and delayed primary closure of contaminated soft tissue injuries in Iraq. *J Trauma*. 2006;61:1207 1211.
8. Labler L, Trentz O. The use of vacuum assisted closure (VAC) in soft tissue injuries after high energy pelvic trauma. *Langenbecks Arch Surg*. 2007;392:601 609.
9. Dedmond BT, Kortesis B, punger K, et al. The use of negative-pressure wound therapy (NPWT) in the temporary treatment of soft-tissue injuries associated with high-energy open tibial shaft fractures. *J Orthop Trauma*. 2007;21:11 17.
10. Stannard JP, Robinson JT, Anderson ER, et al. Negative pressure wound therapy to treat hematomas and surgical incisions following high-energy trauma. *J Trauma*. 2006;60;1301 1306.
11. Kakagia D, Karadimas E, Drosos G, et al. Vacuum-assisted closure downgrades reconstructive demands in high-risk patients with severe lower extremity injuries. *Acta Chir Plast*. 2009;51:59 64.
12. Crumbley DR, Perciballi JA. Negative pressure wound therapy in a contaminated soft-tissue wound. *J Wound Ostomy Continence Nurs*. 2007;34:507 512.
13. Rispoli DM, Horne BR, Kryzak TJ, Richardson MW. Description of a technique for vacuum-assisted deep drains in the management of

cavitary defects and deep infections in devastating military and civilian trauma. *J Trauma*. 2010;68:1247–1252.
14. Institute of Laboratory Animal Resources, National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press; 1996.
15. Kheirabadi BS, Scherer MR, Estep JS, Dubick MA, Holcomb JB. Determination of efficacy of new hemostatic dressings in a model of extremity arterial hemorrhage in swine. *J Trauma*. 2009;67:450–460.
16. Kheirabadi BS, Mace JE, Terrazas IB, et al. Clot-inducing minerals versus plasma protein dressing for topical treatment of external bleeding in the presence of coagulopathy. *J Trauma*. 2010;69:1062–1073.
17. Ubbink DT, Vermeulen H, Segers P, Goslings JC. Negative pressure therapy for surgical wounds. *Ned Tijdschr Geneeskd*. 2009;153:A365.
18. Kiyokawa K, Takahashi N, Rikimaru H, et al. New continuous negative-pressure and irrigation treatment for infected wounds and intractable ulcers. *Plast Reconstr Surg*. 2007;120:1257–1265.
19. Scherer LA, Shiver S, Chang M, et al. The vacuum assisted closure device: a method of securing skin grafts and improving graft survival. *Arch Surg*. 2002;137:930–933; discussion: 933–934.
20. Lianos S, Danilla S, Barraza C, et al. Effectiveness of negative pressure closure in the integration of split thickness skin grafts: a randomized, double-masked, controlled trial. *Ann Surg*. 2006;61:1207–1211.
21. Zannis J, Angobaldo J, Marks M, et al. Comparison of fasciotomy wound closure using traditional dressing changes and the vacuum-assisted closure device. *Ann Plast Surg*. 2009;62:407–409.
22. Geiger S, McCormick F, Chou R, Wandel AG. War wounds: lessons learned from operation Iraqi freedom. *Plast Reconstr Surg*. 2008;122:146–153.
23. Stannard JP, Volgas DA, Stewart R, McGwin G Jr, Alonso JE. Negative pressure wound therapy after severe open fractures: a prospective randomized study. *J Orthop Trauma*. 2009;23:552–557.
24. Niles SE, McLaughlin DF, Perkins JG, et al. Increased mortality associated with the early coagulopathy of trauma in combat casualties. *J Trauma*. 2008;64:1459–1463; discussion 1463–1465.